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PROVISIONAL APPLICATION
Under Rule 53(c)

Sir:

Herewith is a PROVISIONAL APPLICATION
Title: TREATMENT OF VASCULAR DISEASE WITH RECOMBINANT ADENO-ASSOCIATED VIRUS VECTORS ENCODING APOLIPOPROTEIN A-1 AND APOLIPOPROTEIN A-1 MILANO

Atty. Dkt. 307620 C-M | Client Ref

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Date: April 6, 2004

1. Specification: 12 pages 1A. ☐ Claim: _____ pages 1B ☐ 1 Abstract pages
2. ☐ Specification in non-English language 3. ☐ Drawings: sheet(s)
4. The invention ☐ was ☒ was not made by, or under a contract with, an agency of the U.S. Government.
   If yes, Government agency/contact # = _____
5. ☐ Attached is an assignment and cover sheet. Please return the recorded assignment to the undersigned.
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8. This application is made by the following named inventor(s) **(Double check instructions for accuracy.):**

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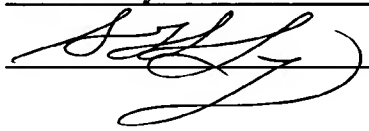
Sig: 

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: PREDIMAN K. SHAH, ET AL. Serial No.: UNASSIGNED Filed: APRIL 6, 2004 For: TREATMENT OF VASCULAR DISEASE WITH RECOMBINANT ADENO-ASSOCIATED VIRUS VECTORS ENCODING APOLIPOPROTEIN A-1 AND APOLIPOPROTEIN A-1 MILANO	Group No.: UNKNOWN Examiner: UNKNOWN

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APPLICATION FOR A
PROVISIONAL UNITED STATES PATENT
IN THE NAMES OF

PREDIMAN K. SHAH, SASWATI CHATTERJEE AND K. K. WONG, JR.

for

TREATMENT OF VASCULAR DISEASE WITH RECOMBINANT ADENO-ASSOCIATED VIRUS VECTORS ENCODING APOLIPOPROTEIN A-1 AND APOLIPOPROTEIN A-1 MILANO

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TREATMENT OF VASCULAR DISEASE WITH RECOMBINANT ADENO-ASSOCIATED VIRUS VECTORS ENCODING APOLIPOPROTEIN A-1 AND APOLIPOPROTEIN A-1 MILANO

FIELD OF THE INVENTION

The invention relates to the treatment of vascular disease, and, more particularly, to the treatment of vascular disease with Apolipoprotein A-1 ("ApoA-1") or ApoA-1 Milano.

BACKGROUND OF THE INVENTION

A host of vascular diseases relate to atherosclerosis, a condition in which fatty material accumulates along the walls of the arteries (*e.g.*, coronary arteries, carotid arteries, aorta, ileofemoral arteries). Over time, this material thickens, hardens and may eventually block or severely narrow the arteries.

ApoA-1, a major component of high density lipoprotein ("HDL"), has been shown to have anti-atherogenic properties. Recently, an Arg173 to Cys point mutation known as ApoA-1 Milano has demonstrated efficacy in both the prevention and treatment of atherosclerotic lesions in murine and rabbit animal models, with potency greater than that of wild type ApoA-1. A recent human trial testing recombinant ApoA-1 Milano infusions has similarly shown significant and rapid reduction in coronary atheroma volumes.

Gene delivery is a promising method for the treatment of acquired and inherited diseases. A number of viral-based systems for gene transfer purposes have been described, such as retroviral systems, which are currently the most widely used viral vector systems for gene transfer. For descriptions of various retroviral systems, see, *e.g.*, U.S. Patent No. 5,219,740; Miller and Rosman (1989) *BioTechniques* 7:980-990; Miller, A.D. (1990) *Human Gene Therapy* 1:5-14; Scarpa *et al.* (1991) *Virology* 180:849-852; Burns *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:8033-8037; and Boris-Lawrie and Temin (1993) *Cur. Opin. Genet. Develop.* 3:102-109. However, the recent description of retrovirus vector-associated leukemogenesis in two patients has underscored potential limitations of this vector system.

A number of adenovirus-based gene delivery systems have also been developed.

Human adenoviruses are double-stranded DNA viruses which enter cells by receptor-mediated endocytosis. These viruses are particularly well suited for gene transfer because they are easy to grow and manipulate and they exhibit a broad host range both *in vivo* and *in vitro*. Adenovirus is easily produced at high titers and is stable so that it can be purified and stored. For descriptions of various adenovirus-based gene delivery systems, see, *e.g.*, Haj-Ahmad and Graham (1986) *J. Virol.* 57:267-274; Bett *et al.* (1993) *J. Virol.* 67:5911-5921; Mittereder *et al.* (1994) *Human Gene Therapy* 5:717-729; Seth *et al.* (1994) *J. Virol.* 68:933-940; Barr *et al.* (1994) *Gene Therapy* 1:51-58; Berkner, K.L. (1988) *BioTechniques* 6:616-629; and Rich *et al.* (1993) *Human Gene Therapy* 4:461-476.

The construction of recombinant adeno-associated virus ("rAAV") vectors has been described. See, *e.g.*, U.S. Patent Nos. 5,173,414 and 5,139,941; International Patent Publication Numbers WO 92/01070 (published Jan. 23, 1992) and WO 93/03769 (published Mar. 4, 1993); Lebkowski *et al.* (1988) *Molec. Cell. Biol.* 8:3988-3996; Vincent *et al.* (1990) *Vaccines* 90 (Cold Spring Harbor Laboratory Press); Carter, B.J. (1992) *Current Opinion in Biotechnology* 3:533-539; Muzyczka, N. (1992) *Current Topics in Microbiol. and Immunol.* 158:97-129; and Kotin, R.M. (1994) *Human Gene Therapy* 5:793-801.

Eukaryotic vectors based upon the nonpathogenic parvovirus, adeno-associated virus ("AAV"), have recently emerged as promising vehicles for efficient gene transfer. AAV is a replication-defective DNA virus with a 4.7 kb genome with palindromic inverted terminal repeats ("ITR"). Coinfection with a helper virus, typically adenovirus or herpes simplex virus, is required for productive infection. In the absence of helper virus coinfection, AAV stably integrates via the ITRs into chromosomal DNA, or may persist in an episomal state. Wild type AAV is unique in the capacity for integration into a specific region of human DNA termed "AAVS1" on human chromosome 19.

The AAV2 genome has two major open reading frames ("ORFs"); the left encodes functions necessary for AAV ori mediated replication and site specific integration (*Rep*), while the right encodes functions necessary for encapsidation (*Cap*). AAV vectors transduce many different types of cells. Multiple studies have amply demonstrated that rAAV vectors can transduce quiescent, nonproliferating targets. rAAV

vectors do not encode any viral encoded genes, reducing their intrinsic immunogenicity. In addition, prolonged *in vivo* transgene expression following rAAV transduction has been documented in animal models. Finally, since its discovery in the mid-1960s, wild type AAV has yet to be definitively identified as a pathogen in either animals or humans. On the contrary, there is evidence that infection with wild type AAV inhibits transformation by bovine and human papillomaviruses and the activated H-ras oncogene *in vitro*, and induces apoptosis in p53 deficient, malignant cells, while epidemiologic studies suggest that prior infection in humans may actually confer an oncoprotective effect. Thus, for reasons outlined above and supported by data described herein, AAV-based vectors are well suited for the stable introduction of transgenes into hematopoietic cells.

There is a need in the art for a gene therapeutic approach to the treatment of atherosclerosis and the array of diseases and physiological conditions related to the same. A therapy that makes use of ApoA-1 and/or ApoA-1 Milano, particularly when delivered via rAAV vector technology, is especially desirable.

DESCRIPTION OF THE INVENTION

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

"Gene transfer" or "gene delivery" refers to methods or systems for reliably inserting foreign DNA into host cells. Such methods can result in transient expression of non-integrated transferred DNA, extrachromosomal replication and expression of transferred replicons (*e.g.*, episomes), or integration of transferred genetic material into the genomic DNA of host cells. Gene transfer provides a unique approach for the treatment of acquired and inherited diseases. A number of systems have been developed for gene transfer into mammalian cells. See, *e.g.*, U.S. Patent No. 5,399,346.

"Vector" refers to any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc., which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

"AAV vector" refers to any vector derived from any adeno-associated virus

serotype, including, without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-7 and AAV-8. AAV vectors can have one or more of the AAV wild-type genes deleted in whole or in part, preferably the *Rep* and/or *Cap* genes, but retain functional flanking ITR sequences. Functional ITR sequences are generally necessary for the rescue, replication, packaging and potential chromosomal integration of the AAV genome. Thus, an AAV vector is defined herein to include at least those sequences required in *cis* for replication and packaging (*e.g.*, functional ITRs) of the virus. The ITRs need not be the wild-type nucleotide sequences, and may be altered (*e.g.*, by the insertion, deletion or substitution of nucleotides) so long as the sequences provide for functional rescue, replication and packaging.

"Recombinant virus" refers to a virus that has been genetically altered (*e.g.*, by the addition or insertion of a heterologous nucleic acid construct into the particle).

"AAV virion" refers to a complete virus particle, such as a wild-type ("wt") AAV virus particle (*i.e.*, including a linear, single-stranded AAV nucleic acid genome associated with an AAV capsid protein coat). In this regard, single-stranded AAV nucleic acid molecules of either complementary sense (*i.e.*, "sense" or "antisense" strands) can be packaged into any one AAV virion; both strands are equally infectious.

A "recombinant AAV virion" or "rAAV virion" is defined herein as an infectious, replication-defective virus composed of an AAV protein shell, encapsidating a heterologous DNA molecule of interest (*e.g.*, genes encoding ApoA-1, ApoA-1 Milano) which is flanked on both sides by AAV ITRs. A rAAV virion may be produced in a suitable host cell which has had an AAV vector, AAV *Rep* and *Cap* functions and helper virus functions introduced therein. In this manner, the host cell is rendered capable of producing AAV replication and capsid proteins that are required for replicating and packaging the AAV vector (*i.e.*, containing a recombinant nucleotide sequence of interest) into recombinant virion particles for subsequent gene delivery. The complete transgene may consist of a promoter, the coding sequences, usually a cDNA and a polyadenylation signal. A transgene may also include regulatory sequences and intron regions. Promoters that would regulate transgene expression may include constitutive, inducible and tissue-specific promoters.

The term "transfection" is used herein to refer to the uptake of foreign DNA by a

cell. A cell has been "transfected" when exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are generally known in the art. See, *e.g.*, Graham *et al.* (1973) *Virology*, 52:456, Sambrook *et al.* (1989) *Molecular Cloning, a laboratory manual*, Cold Spring Harbor Laboratories, New York, Davis *et al.* (1986) *Basic Methods in Molecular Biology*, Elsevier, and Chu *et al.* (1981) *Gene* 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties, such as a plasmid vector and other nucleic acid molecules, into suitable host cells. The term refers to both stable and transient uptake of the genetic material.

The term "transduction" denotes the delivery of a DNA molecule to a recipient cell either *in vivo* or *in vitro*, via any method of gene delivery, including replication-defective viral vectors, such as via a rAAV.

The term "heterologous," as it relates to nucleic acid sequences such as gene sequences and control sequences, denotes sequences that are not normally joined together and/or are not normally associated with a particular virus. Allelic variation or naturally occurring mutational events do not give rise to heterologous DNA, as used herein.

"DNA" is meant to refer to a polymeric form of deoxyribonucleotides (*i.e.*, adenine, guanine, thymine and cytosine) in double-stranded or single-stranded form, either relaxed or supercoiled. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes single- and double-stranded DNA found, *inter alia*, in linear DNA molecules (*e.g.*, restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the non-transcribed strand of DNA (*i.e.*, the strand having the sequence homologous to the mRNA). The term captures molecules that include the four bases adenine, guanine, thymine and cytosine, as well as molecules that include base analogues which are known in the art.

A "gene" or "coding sequence" or a sequence which "encodes" a particular protein is a nucleic acid molecule that is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences; although one of skill in the art will readily

appreciate that various polynucleotides do not operate in this fashion (*e.g.*, antisense RNA, siRNA, ribozymes, wherein the RNA transcript is the product). With respect to protein products (*i.e.*, not RNA products), the boundaries of the coding sequence are determined by a start codon at the 5' (*i.e.*, amino) terminus and a translation stop codon at the 3' (*i.e.*, carboxy) terminus. A gene can include, but is not limited to, cDNA from prokaryotic or eukaryotic mRNA, genomic DNA sequences from prokaryotic or eukaryotic DNA, and even synthetic DNA sequences. A transcription termination sequence will usually be located 3' to the gene sequence. Moreover, a "gene" (i) starts with a promoter region containing multiple regulatory elements, possibly including enhancers, for directing transcription of the coding region sequences; (ii) includes coding sequences, which start at the transcriptional start site that is located upstream of the translational start site and ends at the transcriptional stop site, which may be quite a bit downstream of the stop codon (a polyadenylation signal is usually associated with the transcriptional stop site and is located upstream of the transcriptional stop); and (iii) may contain introns and other regulatory sequences to modulate expression and improve stability of the RNA transcript.

The term "control elements" refers collectively to promoter regions, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites ("IRES"), enhancers, and the like, which collectively provide for the replication, transcription and translation of a coding sequence in a recipient cell. Not all of these control elements need always be present, so long as the selected coding sequence is capable of being replicated, transcribed and translated in an appropriate host cell.

The term "promoter region" is used herein in its ordinary sense to refer to a nucleotide region including a DNA regulatory sequence, wherein the regulatory sequence is derived from a gene which is capable of binding RNA polymerase and initiating transcription of a downstream (3'-direction) coding sequence.

"Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, control elements operably linked to a coding sequence are capable of effecting the expression of the coding sequence. The control elements need not be contiguous with the coding sequence,

so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

For the purpose of describing the relative position of nucleotide sequences in a particular nucleic acid molecule throughout the instant application, such as when a particular nucleotide sequence is described as being situated "upstream," "downstream," "5'," or "3'" relative to another sequence, it is to be understood that it is the position of the sequences in the non-transcribed strand of a DNA molecule that is being referred to as is conventional in the art.

"Homology" as used herein refers to the percent of identity between two polynucleotide or two polypeptide moieties. The correspondence between the sequence from one moiety to another can be determined by techniques known in the art. For example, homology can be determined by a direct comparison of the sequence information between two polypeptide molecules by aligning the sequence information and using readily available computer programs. Alternatively, homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s), and size determination of the digested fragments. Two DNA or two polypeptide sequences are "substantially homologous" to each other when at least about 80%, preferably at least about 90%, and most preferably at least about 95% of the nucleotides or amino acids, respectively, match over a defined length of the molecules, as determined using the methods above.

"Isolated" as used herein when referring to a nucleotide sequence, refers to the fact that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. Thus, an "isolated nucleic acid molecule which encodes a particular polypeptide" refers to a nucleic acid molecule that is substantially free of other nucleic acid molecules that do not encode the subject polypeptide. However, the molecule may include some additional bases or moieties that do not deleteriously affect the basic characteristics of the composition.

"Mammal" as used herein refers to any member of the class Mammalia,

including, without limitation, humans and nonhuman primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs, and the like. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be included within the scope of this term.

The methods of the present invention relate to the treatment, prevention, inhibition, stabilization and/or induction of regression of atherosclerosis, as well as the treatment, prevention, inhibition and/or stabilization of any disease or physiological condition in which atherosclerosis (or atherogenesis) plays a role. Furthermore, the methods of the present invention may be particularly useful in treating atherosclerosis when caused by invasive techniques, such as percutaneous transluminal coronary angioplasty ("PTCA"), insertion of a bypass graft or stent insertion; treatment of restenosis following stent placement or as a result of bypass graft insertion is contemplated as being within the scope of the present invention, as well. Other diseases and physiological conditions that may benefit from the methods of the present invention will be readily apparent to those of skill in the art, and are also contemplated as being within the ambit of the present invention.

The invention is based on a gene therapeutic approach to the treatment of vascular disease. In one embodiment of the invention, rAAV virions encoding heterologous DNA corresponding to an ApoA-1 or ApoA-1 Milano coding sequence are generated by any conventional technique known in the art. By way of example, the recombinant AAV virions of the present invention, including the ApoA-1 or ApoA-1 Milano DNA of interest, can be produced by a standard methodology that generally involves the steps of: (1) introducing an AAV vector plasmid into a host cell; (2) introducing an AAV helper construct into the host cell, where the helper construct includes AAV coding regions capable of being expressed in the host cell to complement AAV helper functions missing from the AAV vector; (3) introducing one or more helper viruses and/or accessory function vectors into the host cell, wherein the helper virus and/or accessory function vectors provide accessory functions capable of supporting efficient rAAV virion

production in the host cell; and (4) culturing the host cell to produce rAAV virions. The AAV vector, AAV helper construct and the helper virus or accessory function vector(s) can be introduced into the host cell either simultaneously or serially, using standard transfection techniques.

AAV vectors are constructed using known techniques to at least provide, as operatively linked components in the direction of transcription, (a) control elements including a transcriptional initiation region, (b) the ApoA-1 or ApoA-1 Milano DNA of interest and (c) a transcriptional termination region. Moreover, any coding sequence sufficiently homologous to the ApoA-1 or ApoA-1 Milano coding sequence so as to exhibit functional properties substantially similar to the ApoA-1 or ApoA-1 Milano coding sequence may be used in connection with alternate embodiments of the present invention. The control elements are selected to be functional in the targeted cell(s). The resulting construct, which contains the operatively linked components, may be bounded (5' and 3') with functional AAV ITR sequences. The nucleotide sequences of AAV ITR regions are known. See, *e.g.*, Kotin, R.M. (1994) *Human Gene Therapy* 5:793-801; Berns, K.I. "Parvoviridae and their Replication" in *Fundamental Virology*, 2nd Edition, (B.N. Fields and D.M. Knipe, eds.) for the AAV-2 sequence. AAV ITRs used in the vectors of the invention need not have a wild-type nucleotide sequence, and may be altered (*e.g.*, by the insertion, deletion or substitution of nucleotides). Additionally, AAV ITRs may be derived from any of several AAV serotypes, including, without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-7, AAV-8 and the like. Furthermore, 5' and 3' ITRs that flank a selected nucleotide sequence in an AAV expression vector need not necessarily be identical or derived from the same AAV serotype or isolate, so long as they function as intended (*i.e.*, to allow for excision and replication of the bounded ApoA-1 or ApoA-1 Milano nucleotide sequence of interest).

The rAAV genome (usually AAV-2) encoding the Apo A-1 or Apo A-1 Milano transgenes within AAV ITRs may be packaged in virion capsids derived from any AAV serotype including AAV-1, AAV-2, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8 and the like.

In accordance with an embodiment of the invention, the rAAV virions including an ApoA-1 or ApoA-1 Milano coding sequence are delivered to a mammal in a sufficient

quantity and by a sufficient delivery route so as to effect gene transfer. As described in the ensuing Examples, this provides an effective treatment for atherosclerosis in mammals. In one embodiment of the present invention, the rAAV-ApoA-1 or rAAV-ApoA-1 Milano vector may be delivered to a subject by first transducing hematopoietic stem/progenitor cells (*e.g.*, bone marrow cells) with a quantity of the rAAV-ApoA-1 Milano vector, and then transplanting these cells into a mammal. In an alternate embodiment, the rAAV-ApoA-1 Milano vector may be introduced into a mammal by direct intramuscular or intravenous injection, or directly into the artery at the site of PTCA or stent placement by any conventional methodology, as will be readily appreciated by one of skill in the art. This results in secretion of ApoA-1 Milano either directly into the circulation or locally in atherosclerotic plaque areas.

While the description above refers to particular embodiments of the present invention, it will be understood that many modifications may be made without departing from the spirit thereof. The presently disclosed embodiments and ensuing Examples are therefore to be considered in all respects as illustrative and not restrictive.

EXAMPLES

The following Examples demonstrate the success of gene therapy in treating atherosclerosis using rAAV vectors encoding ApoA-1 Milano. The Examples further demonstrate the success of these vectors, *in vivo*, following either intramuscular injection or transplantation of rAAV-transduced hematopoietic progenitor cells.

EXAMPLE 1

rAAV-2 and rAAV-5 Vectors Encoding ApoA-1 Milano Demonstrate Anti-atherogenic Properties in Animal Model

The anti-atherogenic properties of rAAV vectors encoding ApoA-1 Milano were tested in homozygous transgenic Apolipoprotein E ("ApoE") -/- mice. These mice develop large vessel atherosclerotic plaques when fed a high fat diet. Foam cells, which play a pivotal role in atherogenesis, arise from bone marrow-derived macrophages, and transplantation with wild type bone marrow cells has been shown to prevent the onset of

atherosclerosis in ApoE-/- mice. In this study the hypothesis that transplantation of transduced bone marrow cells would protect against the development of atherosclerosis was tested. The efficacy of transplantation with ApoA-1 Milano transduced bone marrow cells in reducing the extent of atherosclerosis was compared with direct intramuscular vector injection. Direct intramuscular vector injection results in secretion of ApoA-1 Milano directly into the circulation.

For the transplants, ten million ApoE-/- bone marrow cells were transduced overnight with rAAV-ApoA-1 Milano at a multiplicity of infection (moi) of 5000, washed and infused via the tail vein into 6-8 week old, lethally irradiated male Apo E-/- mice. For the intramuscular injections, 6-8 week old ApoE-/- mice were injected with 3-$5x10^{12}$ vector genomes/kg. A high fat diet was initiated two weeks after the procedures. Approximately 20-24 weeks later, the aorta and large vessels extending from the aortic arch to femoral bifurcation were isolated, stained with oil red O, and the extent of atherosclerotic plaques were quantified. Negative controls included transplantation of cells transduced with an irrelevant rAAV, untransduced transplants and unmanipulated mice. Positive controls included transplantation of wild type C57BL6/J bone marrow cells into irradiated ApoE-/- mice.

Although all ApoA-1 Milano treated groups showed marked reductions in plaque formation, transplantation with rAAV/Apo A-1 Milano-transduced marrow and intramuscular injection of rAAV-ApoA-1 Milano resulted in a significant (approx. 50-60% reduction in aortic atherosclerotic plaque formation as compared with controls. These results suggest that transplantation of rAAV-Apo A-1 Milano transduced hematopoietic progenitor cells and direct intramuscular injection may provide a novel and efficient strategy for controlling the development of atherosclerosis.

EXAMPLE 2

Long-term Inhibition of Atherogenesis Demonstrated in Animal Model by Intramuscular Injection or Transplantation of rAAV-Transduced Bone Marrow Cells

The effectiveness of rAAV vectors encoding ApoA-1 Milano for gene therapy of atherosclerosis *in vivo* was tested. In particular, the effectiveness of these vectors following either intramuscular injection or transplantation of rAAV-transduced bone

marrow cells was examined.

ApoE-/- mice were injected with approximately 10^{12} vector genomes/kg at 6-8 weeks of age and placed on a high fat diet two weeks later. No major differences in body weight were observed between the treatment groups. 20-24 weeks after injection, the mice were harvested, aortas were cleaned, fixed, mounted, stained with oil red O and atherosclerotic plaque areas quantified. Control untreated mice or mice treated with an irrelevant rAAV vector showed marked atherosclerotic lesion formation. In contrast, the rAAV-2-ApoA-1 Milano and rAAV-5-ApoA-1 Milano injected groups showed significant reductions in plaque formation, 58% and 50% respectively, despite a lack of major differences in total plasma cholesterol levels.

Because marrow-derived monocytes and macrophages play a pivotal role in atherogenesis, it was hypothesized that transplantation of rAAV-ApoA-1 Milano-transduced marrow cells would also result in a decline in plaque formation, possibly due to the localization of transduced macrophages within atherosclerotic lesions.

Lethally irradiated ApoE-/- mice were transplanted with rAAV transduced cells, and high fat diet was initiated two weeks later. 22 to 24 weeks after transplantation, the mice were euthanized, and aortas were analyzed for atherosclerotic plaques. Untransplanted mice, and mice transplanted with untransduced and control rAAV-transduced marrow served as negative controls. Wild type B6 to ApoE-/- transplants served as positive controls for plaque reduction. Results revealed a 47% and 49% reduction in atheroma formation following transplantation with marrow cells transduced with rAAV-2 and rAAV-5 vectors encoding ApoA-1 Milano, respectively.

These results suggest that intramuscular injection of rAAV-ApoA-1 Milano or transplantation of rAAV-transduced marrow cells results in significant long-term inhibition of atherogenesis following a single treatment with an rAAV vector.

ABSTRACT

Described herein is a gene therapeutic approach to the treatment of atherosclerosis. The inventive methods may be used in the treatment of atherosclerosis, as well as any disease or physiological condition in which atherosclerosis plays a role. The inventive methods involve the gene delivery of ApoA-1 or ApoA-1 Milano. This may be accomplished by the use of rAAV technology. rAAV virions may be delivered to a mammalian subject by various methodologies, including transplantation of transduced bone marrow cells, direct intramuscular injection, intravenous or portal vein injection or stent delivery.